

Amendments to the Claims

This listing of claims will replace all prior versions, and listing, of claims in the application:

Listing of Claims:

1.-2. (Canceled)

3. (Previously presented) A nucleic acid ligation assay, comprising:

contacting a sample suspected of containing one or more target nucleic acid sequences with one or more subsets of free probes and one or more subsets of spectrally-addressable bound probes;

allowing the one or more subsets of free probes and the one or more subsets of spectrally-addressable bound probes to hybridize to the one or more target nucleic acid sequences, if present in the sample;

ligating the hybridized free probes with the hybridized spectrally-addressable bound probes, wherein a free probe hybridized to a target nucleic acid sequence is ligated with a spectrally-addressable bound probe hybridized to the same target nucleic acid sequence to provide spectrally-addressable ligated products; and

detecting a presence of the spectrally-addressable ligated products, analyzing the target nucleic acid sequences of the spectrally-addressable ligated products, or performing said detecting and said analyzing; and wherein:

the one or more target nucleic acid sequences comprise one or more first and one or more second target nucleic acid sequences, wherein the one or more first target nucleic acid sequences have at least a first portion and a second portion, wherein the one or more second target nucleic acid sequences have at least a first portion and a second portion, wherein the first portion of the one or more first target nucleic acid sequences is distinguishable from the first portion of the one or more second target nucleic acid sequences, and wherein the second portion of the one or more first target nucleic acid sequences is substantially identical to the second portion of the one or more second target nucleic acid sequences; and

the one or more subsets of spectrally-addressable bound probes comprise a first subset and a second subset, wherein the first subset is specific for the first portion of the one or more first target nucleic acid sequences, wherein the second subset is specific for the first portion of the one or more second target nucleic acid sequences, and wherein the one or more subsets of free probes have substantially identical nucleotide sequences that are specific for the second portion of the one or more first and second target nucleic acid sequences.

4. (Canceled)

5. (Currently amended) A nucleic acid ligation assay, comprising:

contacting a sample suspected of containing one or more target nucleic acid sequences with one or more subsets of free probes and one or more subsets of spectrally-addressable bound probes;

allowing the one or more subsets of free probes and the one or more subsets of spectrally-addressable bound probes to hybridize to the one or more target nucleic acid sequences, if present in the sample;

ligating the hybridized free probes with the hybridized spectrally-addressable bound probes, wherein a free probe hybridized to a target nucleic acid sequence is ligated with a spectrally-addressable bound probe hybridized to the same target nucleic acid sequence to provide spectrally-addressable ligated products; and

detecting a presence of the spectrally-addressable ligated products, analyzing the target nucleic acid sequences of the spectrally-addressable ligated products, or performing said detecting and said analyzing; and wherein:

the one or more target nucleic acid sequences comprise one or more first and one or more second target nucleic acid sequences, wherein the one or more first target nucleic acid sequences have at least a first portion and a second portion, wherein the one or more second target nucleic acid sequences have at least a first portion and a second portion, wherein the first portion of the one or more first target nucleic acid sequences is distinguishable from the first portion of the one or more second target nucleic acid

sequences, and wherein the second portion of the one or more first target nucleic acid sequences is substantially identical to the second portion of the one or more second target nucleic acid sequences; and

the one or more subsets of free probes comprise a first subset and a second subset, wherein the one or more subsets of spectrally-addressable bound probes comprise ~~one~~ a third subset, wherein the first subset is specific for the first portion of the one or more first target nucleic acid sequences, wherein the second subset is specific for the first portion of the one or more second target nucleic acid sequences, and wherein the ~~one-third~~ subset has substantially identical nucleotide sequences that are specific for the second portion of the one or more first and second target nucleic acid sequences.

6. (Previously presented) An assay according to claim 5, wherein the assay is performed in a first and a second reaction vessel, wherein a first portion of the sample is contacted with the first subset in the first reaction vessel, and wherein a second portion of the sample is contacted with the second subset in the second reaction vessel.

7. (Previously presented) An assay according to claim 3, further comprising using a thermostable ligase for said ligating.

8. (Previously presented) An assay according to claim 3, wherein each of the spectrally-addressable bound probes in the first subset comprise a first amount of at least one fluorescent dye, wherein each of the spectrally-addressable bound probes in the second subset comprise a second amount of the at least one fluorescent dye, wherein the first amount is different than the second amount, and wherein the first subset and the second subset are distinguishable based at least on the first and second amounts.

9. (Previously presented) An assay according to claim 3, further comprising contacting the sample with polymerase chain reaction components and amplifying the one or more target nucleic acid sequences.

10. (Currently amended) A microsphere-based oligonucleotide ligation assay, comprising:

(a) contacting a sample, which is suspected of containing target nucleic acid molecules, with a mixture comprising ~~at least one~~ a subset of free probes and ~~at least one~~ a subset of bound probes, wherein:

(i) the free probes ~~of the at least one subset of free probes~~ comprise two opposing ends, wherein a detectable label is at a first of the two opposing ends, wherein a nucleotide is at a second of the two opposing ends, and wherein the free probes ~~of the at least one subset of free probes~~ further comprise an oligonucleotide having a predetermined nucleotide sequence that is complementary to at least a first portion of the target nucleic acid molecules;

(ii) the bound probes ~~of the at least one subset of bound probes~~ comprise a microsphere and an oligonucleotide probe, wherein the oligonucleotide probes comprise an oligonucleotide at a first end of the oligonucleotide probes having a modifier moiety, which is used for coupling the oligonucleotide probes to the microspheres, and wherein the oligonucleotide probes further comprise an oligonucleotide having a predetermined nucleotide sequence that is complementary to at least a second portion of the target nucleic acid molecules; and

(iii) the microspheres of ~~each subset of the at least one subset of bound probes~~ having an unique spectral address or an unique fluorescence intensity, which distinguishes the microspheres of ~~different the subset of bound probes of the at least one from an additional subset of bound probes~~;

(b) allowing the ~~at least one subset of free probes~~ and the ~~at least one subset of bound probes~~ to hybridize to the target nucleic acid molecules;

(c) ligating one ~~end of the two opposing ends~~ of the oligonucleotides of the hybridized free probes with one end of the oligonucleotides of the hybridized bound probes to provide microsphere-bound ligated products; and

(d) detecting a ~~the~~ presence of the microsphere-bound ligated products.

11. (Currently amended) The assay of Claim 10, wherein the ~~at least one subset of free probes~~ and the ~~at least one subset of bound probes~~ hybridize to different portions of the target nucleic acid molecules.

12. (Previously presented) The assay of claim 11, wherein the different portions of the target nucleic acid molecules do not overlap.

13. (Currently amended) The assay of claim 10, wherein the free probes ~~of the at least one subset of free probes~~ further comprise a phosphate at ~~the one end of the oligonucleotides of the free probes.~~

14. (Currently amended) The assay of claim 10, wherein the bound probes ~~of the at least one subset of bound probes~~ further comprise a phosphate at ~~the one end of the oligonucleotides of the bound probes.~~

15. (Currently amended) The assay of claim 10, ~~wherein the at least one subset of bound probes comprises at least two subsets~~ wherein the mixture further comprises the additional subset of bound probes, wherein the oligonucleotide probes coupled to the microspheres of the ~~at least two subsets~~ subset of bound probes are different ~~than oligonucleotide probes coupled to microspheres of the additional subset.~~

16. (Currently amended) The assay of claim 15, wherein a first nucleotide of the predetermined nucleotide sequences of the ~~at least two subsets~~ subset of bound probes ~~are~~ is different than a first nucleotide of predetermined nucleotide sequences of the additional subset of bound probes, and wherein other nucleotides of the predetermined nucleotide sequences of the ~~at least two subsets~~ subset of bound probes are substantially identical to ~~other nucleotides of the predetermined nucleotide sequences of the additional subset of bound probes.~~

17. (Currently amended) The assay of claim 16, wherein the free probes ~~of the at least one subset of free probes~~ have substantially identical predetermined nucleotide sequences.

18. (Currently amended) The assay of claim 15, wherein identities of one or more nucleotides at one or more positions of the predetermined nucleotide sequences of the ~~at least two subsets~~ subset of bound probes are different ~~than identities of one or more nucleotides at the one or more positions of predetermined nucleotide sequences of the additional subset of bound probes.~~

19. (Currently amended) The assay of claim 15, wherein the ~~at least two subsets~~ subset of bound probes have differ due to one or more substitutions, insertions, deletions, or combinations thereof, at one or more positions of the predetermined nucleotide sequences ~~that differ from the one or more substitutions, insertions, deletions, or combinations thereof at the one or more positions of predetermined nucleotide sequences of the additional subset of bound probes.~~

20. (Currently amended) The assay of claim 11, ~~wherein the at least one subset of free probes comprises at least two subsets wherein the mixture further comprises an additional subset~~ subsets of free probes, wherein the nucleotide and the detectable label of a first subset of the at least two subsets ~~the subset~~ of free probes differ from ~~the a nucleotide and the a detectable label of a second subset of the at least two subsets of free probes~~ the additional subset of free probes, and wherein the predetermined nucleotide sequence ~~sequence of the first and second subsets~~ subset of free probes is ~~are~~ substantially identical to a predetermined nucleotide sequence of the additional subset of free probes.

21. (Currently amended) The assay of claim 20, wherein the bound probes ~~of the at least one subset of bound probes~~ have substantially identical predetermined nucleotide sequences.

22. (Currently amended) The assay of claim 10, wherein the oligonucleotides of the ~~at least one subset of free probes and the at least one subset of bound probes~~ have 5' and 3' ends, wherein the free probes ~~of the at least one subset of free probes include~~ further comprise a phosphate at the 5' ends and the detectable label at the 3' ends, and wherein the modifier moiety is an amine which couples the 5' end of the oligonucleotide of ~~one the bound probe~~ probes to a carboxylic acid group on the microsphere of the ~~one bound probe~~ probes.

23. (Currently amended) The assay of claim 22, ~~wherein the at least one subset of bound probes comprises at least two subsets wherein the mixture further comprises the additional subset of bound probes, and wherein the oligonucleotides coupled to the microspheres of the at least two subsets~~ subset of bound probes are different than oligonucleotides coupled to microspheres of the additional subset of bound probes in that:

a portion of the 3' ends of the oligonucleotides of the ~~at least two subsets~~ subset of bound probes and the additional subset of bound probes differs in nucleotide sequence; and

~~wherein other portions of the 3' ends of the oligonucleotides of the at least two subsets~~ subset of bound probes and the additional subset of bound probes have nucleotide sequences that are substantially identical.

24. (Currently amended) The assay of claim 22, ~~wherein the at least one subset of free probes comprise at least two subsets wherein the mixture further comprises an additional subset of free probes, wherein a portion of the oligonucleotides found at the 5' ends of the at least two setssubset of free probes is different than a portion of oligonucleotides found at 5' ends of the additional subset of free probes, and wherein other portions of the nucleotide sequences-sequence of the oligonucleotides of the at least two subsetssubset of free probes are substantially identical to other portions of a nucleotide sequence of the additional subset of free probes.~~

25. (Previously presented) The assay of claim 22, wherein the assay is carried out in a single reaction vessel.

26. (Currently amended) The assay of claim 22, ~~wherein the mixture further comprises an additional subset of free probes, wherein the assay is carried out in separate reaction vessels, using at least one of the separate reaction vessel-vessels for each of the at least one subset of free probes and the additional subset of free probes.~~

27. (Currently amended) The assay of claim 23, wherein the microspheres of the ~~at least two subsetssubset of bound probes harbor at least one fluorescent dye that emits, upon exposure to an excitatory stimulus, signals having different intensities that are different than intensities of signals emitted, upon exposure to the excitatory stimulus, by the at least one fluorescent dye of the additional subset of bound probes.~~

28. (Currently amended) The assay of claim 23, wherein the microspheres of the ~~at least two subsetssubset of bound probes have different relative amounts of at least two fluorescent dyes that are different than relative amounts of the at least two fluorescent dyes of the microspheres of the additional subset of bound probes.~~

29. (Previously presented) The assay of claim 22, wherein the mixture further comprises polymerase chain reaction components, and wherein the assay further comprises amplifying a portion of the target nucleic acid molecules.

30.-34. (Canceled)

35. (Previously presented) The assay of claim 10, wherein the modifier moiety comprises an amine modifier moiety.

36. (Currently amended) The assay of claim 10, wherein the modifier moiety comprises a primary amine group for coupling the bound probes ~~of the at least one subset of bound probes~~ to a carboxylic acid group of the microspheres.